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14. ABSTRACT

There is currently no way to predict which patients with NF1 are at high risk for serious complications. We have undertaken an exploratory study of monozygotic (MZ) twins with NF1 who are discordant for various NF complications, and assessed for differences in copy number variations (CNV) in their DNA using genetic microarray technology. We are testing the hypothesis that MZ twin pairs with NF1 will have within-pair differences in CNVs that may explain their discordant NF complications. To date, we have enrolled and studied 10 pairs of MZ twins with NF1 and 12 parents. Preliminary data analysis shows a mean of 19.6 raw CNVs per twin pair, with no de novo CNVs and no discordancies in CNVs within twin pairs found in analysis of the first 5 twin sets. On average, 3.6 CNVs per twin pair contain genes, and could be candidates for modifiers of phenotype. Data analysis is continuing, with emphasis on genes located within CNVs that may affect clinical phenotype.

15. SUBJECT TERMS

Neurofibromatosis 1; monozygotic twins; copy number variation

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INTRODUCTION:

One of the most challenging aspects of managing patients with neurofibromatosis 1 (NF1) is the extreme variability of phenotype. Currently, there is no way to predict which patients are at high risk for serious complications such as malignancy, and to appropriately manage those at higher risk. Even monozygotic twins with NF1 can demonstrate variable expression of the disease. The underlying mechanism(s) for this discordance has never been elucidated, although possibilities proposed have included: modifying genes, stochastic "2nd hit" events, environmental effects, epigenetic alterations, and post-zygotic (somatic) mutations. In this exploratory study, we have evaluated monozygotic twins with NF1 who have concordant and discordant NF features for differences in copy number variants (CNVs). CNVs are microdeletions and micro-duplications of DNA which occur widely throughout the genome, and which may play a significant role in disease predisposition. Study of CNVs in monozygotic twins with NF1 who differ in their manifestations and complications of NF1 may provide us a tool to search for potential mechanisms causing NF1 variability.

BODY:

Task 1 (S.A. 1) Obtain expedited IRB review from local IRB and DOD

IRB approval has been obtained from Cincinnati Children's Hospital and from the HRPO of USAMRMC. Continuing Review report was sent to HRPO on 8/23/11.

Performed by: P.I. student helper, study coordinator.

Task 2 (S.A. 1) Enroll MZ twins and parents, and obtain samples for DNA. (Goal of 11 pairs of twins plus parents)

We have enrolled 10 sets of MZ twins (20 subjects) with NF1 and 12 parents of twins, and have obtained blood and cheek brush (or saliva) samples on each. Samples have not been able to be obtained on all parents, as one set of twins was adopted, and 3 sets of twins had a single parent available.

Performed by: P.I., student assistant, study coordinator.

An additional 4 pairs of twins from other states (Illinois and Utah) have expressed interest in the study, and we are in process of trying to obtain samples from them. Difficulties with the IRB process at these sites has slowed the recruitment of these twins.

We have collected phenotypic data on each of the twins entered, to document their NF1 features and whether they are concordant or discordant within the pair (See Table 1). Twins were found to be highly concordant for overall numbers of café-au-lait spots and cutaneous neurofibromas. They were discordant for optic nerve gliomas and plexiform neurofibromas, implying that a stochastic second hit event as a likely etiology for these tumors. Three pairs of twins were discordant for scoliosis, and one pair was concordant for scoliosis, but differing in degree of severity and need for surgery.

Task 3 (S.A. 1) Isolate DNA from blood and cheek brush of twins, and from blood samples of parents.

This has been accomplished for the 20 twins and 12 parents so far.

Good quality DNA was obtained from all blood samples; however, DNA from cheek brush samples was of unacceptable quality for microarray analysis. Therefore, we substituted saliva samples for cheek brush for the remainder of the study.

Performed by: Molecular genetics lab, CCHMC

Task 4. Interrogate samples using CGH microarray technology

DNA samples from twins and parents were analyzed using Illumina 610-quad BreadChip, SNP-based program. CNVs of >200 kb were validated using FISH.

Microarray has been performed on 36 blood samples from twins and parents; 20 cheek brush samples from twins.

Performed by Dr. Smolarek, cytogenetics laboratory technicians

Task 5. (Sub-aim 1b) Compare CNVs in 2 different tissue types (blood and cheek swab) for each twin.

Performed by: Dr. Smolarek, student helper

DNA quality from cheek swab of initial 10 twins was of unacceptable quality. Therefore, for the remaining twin pairs, we used saliva samples for the second source of DNA.
Analysis of this data will be completed in months 12-18.

Task 6 (Sub-aim 1b) Compare all CNVs found in subjects to those of parents and co-twins
Data analysis has been performed on the first 5 pairs of twins enrolled. CNVs were visually inspected, compared to known polymorphisms from the Database of Genomic Variants, and compared to the co-twin and parents. Conservative CNVs were defined as those with CNV confidence values >100.
Mean number of raw CNVs was 19.6 per patient (range 12-26; see Table 2). A mean of 3.6 CNVs per patient were found to contain genes. All CNVs identified thus far have been concordant within the twin pair, and inherited from a parent. See tables 3 and 4 for chromosomal location of CNVs and for listing of contained genes.
Performed by: P.I., Student helper, Dr. Smolarek
The remaining sets of twins will be analyzed in months 12-24.

Task 7 (Specific Aim 1) Compare CNVs within twin pairs; look for correlation between discordant CNVs and discordant clinical phenotypes in twin pairs.
Detailed phenotypic data has been collected on each twin pair, with concordant and discordant features noted. Prominent discordant features have included: plexiform neurofibromas (number and location), scoliosis, and malignancy (MPNST).
Correlation between CNVs and clinical phenotype will be performed in months 12-24.

Task 8 (Sub-aim 1c). Assess functional significance of identified CNVs.
Preliminary data: Several genes of potential interest were found closely located to CNVs. These included PTPN20A/B, and MAP2K3 in pair A, which are downstream targets of ras. A large duplication in chromosome 17 was found in twin pair A, in trans to NF1 and located 4.1 mB away from the NF1 gene. This is a reported polymorphism in the general population. This twin pair had severe involvement with NF1 with multiple paraspinal tumors and mild cognitive impairment, and were discordant for MPNST. More study of this duplication is indicated to determine if it may predispose to more severe NF1 complications occurring in patients with NF1.
Final analysis will be completed in months 12-24.

Task 9 (Specific Aim 2) Compare the rate of de novo CNVs in individuals with NF1 to published figures for the non-NF1 population.
This task will be performed in months 12-24.

Task 10 Published results in medical literature
Will be performed in months 18-24.

KEY RESEARCH ACCOMPLISHMENTS:

1. Enrolled 10 pairs of MZ twins with NF1 and their parents, collected DNA from blood and cheek brush or saliva. Documented features of NF1 in each pair. Several additional pairs are planned to be added within the next year.
2. Preliminary data shows mean number of 19.6 raw CNVs per twin pair, with no discordancies in CNVs within twins seen so far.
3. CNVs were widely distributed within the genome. There was a predominance of deletions in chromosome 4q, a region where variation is quite common in the general population.
4. Several genes of potential interest were located in close proximity to reported CNVs (PTPN20A/B, MAP2K3), which will be further analyzed for potential effect on NF1 phenotype.

REPORTABLE OUTCOMES: No publications to date from this research. As a result of obtaining this grant, we were able to receive funding for an ancillary project award of $25,000 from the Cincinnati Center for Neurofibromatosis Research (NIH-funded, N. Ratner, P.I.) . This award was to expand the project to perform NF1 sequencing of the same series of twins, to determine if specific genotypes correlated with specific NF1 complications in the twins.

CONCLUSIONS: Evaluation of monozygotic twins can be a valuable tool for studying modifying factors of NF1 features and complications. In this exploratory study of copy number variation in MZ twins with NF1, we have studied MZ twins with NF1 and various concordant and discordant NF features for differences in CNV which could predict specific NF1 complications. We have thus far obtained blood and cheek brush or saliva samples on 10 pairs of twins and 12 parents, with a goal of obtaining an additional 2-3 more sets of twins. From data analysis of the first 5 sets of twins, we have found a mean of 19.6 raw CNVs per patient, with a mean of 3.6 CNVs containing genes per patient. None of the CNVs thus far have been de novo, and we have found no discordant CNVs within twin pairs. Analysis of the remaining twins pairs, and further analysis of functional significance of identified CNVs will occur in year two of the project.

REFERENCES: N/A

APPENDICES: N/A

SUPPORTING DATA: See attached Tables 1-4.

Table 1. Concordant and discordant features of twin pairs (first 5 pairs enrolled) (Concordant features in blue; discordant features in pink).

Twin Pair	A	B	C	D	E
Age (years)	18	10	9	6	5
Gender	F	F	F	F	F
CAL #s	15/15	15/15	20/20	10/9	6/6
Cut. NF	5/5	-/-	-/-	1/1	-/-
Lisch	+/+	+/+	+/+	-/-	-/-
Plexiform #	2/1	-/1	-/-	-/1	-/-
OPG	-/-	+/-	+/-	-/+	-/-
T2 Hyperint	+/+	+/+	+/-	+/+	-/-
Scoliosis	-/-	-/-	-/10°	-/-	-/-
Pectus	+/+	+/-	+/+	-/-	-/-
MPNST	+/-	-/-	-/-	-/-	-/-
MR	-/-	-/-	-/-	-/-	-/-
LD	+/+	+/+	+/+	+/-	-/-
Speech	+/+	-/-	+/+	+/+	+/+
ADHD	+/+	Poss.	-/-	-/-	-/-

Table 2. CNV numbers in 5 MZ twin pairs

Twin Pair	A	B	C	D	E	Total	Mean
Raw CNVs	22	21	17	12	26	98	19.6
Conservative CNVs	11	8	9	3	12	43	8.6
CN Losses	9	8	9	2	9	37	7.4
Polymorphism	8	6	8	3	9	34	6.8
Contain genes	6	3	3	1	5	18	3.6

Table 3: Location of CNVs in 5 pairs of MZ twins

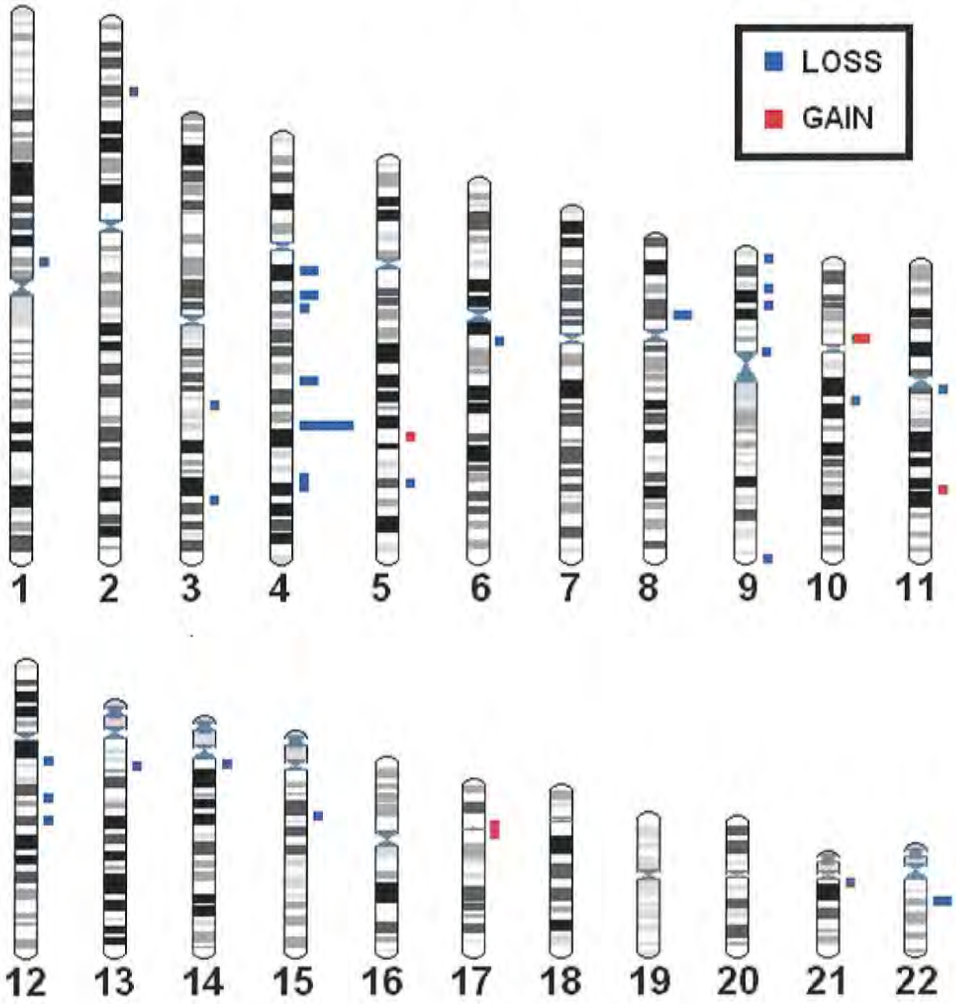

Table 4. Genes located in CNVs in 5 twin pairs

Cyto Band	Genes	CNV type
3q22.1	CPNE4	loss
4q13.2	UGT2B17	loss
4q13.2	UGTUB28	loss
4q31.3	LRBA	loss
5q33.1	predicted	loss
8p11.23	tMDC	loss
8p11.23	tMDC, TMDCII	loss
9q34.3	predicted	**gain**
10q11.22	GPRIN2, ANXA8/L1/L2, SYT15, L25628, predicted	**gain**
10q11.22	predicted	**gain**
11q11	OR4 family	loss
11q22.3	predicted	**gain**
12q15	predicted	loss
15q14	GOLGA8B, predicted	loss
17p11.2-17q11.1	predicted	**gain**
21q21.1	CHODL	loss
22q11.23	GSTT1, predicted	loss
22q11.23	LRP5L, predicted	loss

Table4. Genes within regions of CNV. 18 regions of conservative CNV were found to contain or overlap known and predicted gene sequence; these are summarized here. Predicted may refer to RefSeq or Ensembl genes, or Mammalian Gene Collection mRNA sequences. Cytogenetic location, as well as type (copy number gain/loss) of CNVs are shown.